## AMENDMENT TO THE SPECIFICATION

At the title of the application:

~~Yeast Strains for the Production of Lactic Acid~~ Processes for Producing Lactic Acid Using Yeast Transformed with a Gene Encoding Lactate Dehydrogenase

The paragraph beginning at p. 1, line 1:

This application is a ~~continuation~~ divisional of Application Ser. No. 09/508,277, ~~filed March 7, 2000~~ having a 35 U.S.C. §102(e) date of June 29, 2000, issued as U.S. Pat. No. 6,429,006 on August 6, 2002, which is a 35 U.S.C. §371 national phase entry of PCT/EP98/05758, filed September 11, 1998.

At p. 28, lines 4-15:

The original starting codon (GTG) of the *Lactobacillus casei LDH* gene (GTG) (the LDH sequence is available at the accession ~~n.~~ no. M76708 of the GenBank Sequence Database provided by the National Center of Biotechnology -NCBI- Web site: ~~http://www.ncbi.nlm.nih.gov/~~ http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=149574, accessed February 16, 2004, reporting a sequence first published by Kim, S.F., Baek, S.J. and Pack, M.Y., "Cloning and nucleotide sequence of the Lactobacillus casei lactate dehydrogenase gene," *Appl. Environ. Microbiol.* 57 (8), 2413-2417 (1991)) is not correctly recognised by S. cerevisiae. We obtained plasmid pST2 and LDH sequence from Hutkins Robert, University of Nebraska, USA). pST2 is based on pUC19 vector (Boehringer Mannheim GmbH, Mannheim, Germany, cat. 885827) and

contains a BamHI-SphI LDH-cDNA fragment amplified from the L. casei 686 (Culture collection of the University of Nebraska).

At p. 29, line 22 to p. 30, line 6:

Following a classical PCR approach we also cloned the L(+) LDH genes from the bacteria Bacillus megaterium and Bacillus stearothermophylus (Biol. Chem. Hoppe-Seyler, 1987, 368:1391) (Biol. Chem. Hoppe-Seyler, 1987, 368:1167) (the DNA sequence is also available at the accession ~~n.~~ nos. M22305 and M19396 of the Genbank Sequence Database provided by the National Center of Biotechnology -NCBI- Web site: ~~http://www.ncbi.nlm.nih.gov/~~ http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=143135 and http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=143137, accessed February 16, 2004, reporting sequences first published by Weber, Zuber, and coworkers in Biol. Chem. Hoppe-Seyler, *supra.*) in expression vectors for yeasts S. cerevisiae (i.e., pBME2 and pBST2, respectively-, see below).

At p. 32, line 25 to p. 33, line 8:

The DNA sequence of JEN1 (the DNA sequence is available at the accession ~~n.~~ no. U24155 of the Genbank Sequence Database provided by the National Center of Biotechnology - NCBI- Web site: ~~http://www.ncbi.nlm.nih.gov/~~ http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=780801, accessed February 16, 2004, reporting a sequence first published by E.S. Davis, below), encoding for the lactate transporter of S. cerevisiae (Davis E.S., Thesis, 1994-Laboratory of Eukaryotic Gene Expression,

Advanced Bioscience Laboratories) (Davis, E.S. et al., Proc. Natl. Acad. Sci. U.S.A. 89 (23), 11169, 1992) (Andre, B. Yeast (11), 1575, 1995), has been obtained from E.S. Davis (University of Maryland, USA). The JEN1 coding sequence has been amplified by classical PCR approach described throughout the text and cloned into the pasmid pYX022 (see above). On the integrative plasmid, JEN1 overexpression is under the control of the TPI promoter.

At the table at p. 36, line 1 (the underlining in the right-hand column being original):

| | |
|---|---|
| PDC1-S1 (SEQ ID NO: 5) | TTC TAC TCA TAA CCT CAC GCA AAA TAA CAC AGT CAA ATC ACA GCT GAA GCT TCG TAC GC |
| PDC1-S2 (SEQ ID NO: 6) | AAT GCT TAT AAA ACT TTA ACT AAT AAT TAG AGA TTA AAT CGC ATA GGC CAC TAG TGG ATC TG |
| PDC5-S1 (SEQ ID NO: 7) | ATC AAT CTC AAA GAG AAC AAC ACA ATA CAA TAA CAA GAA GCA GCT GAA GCT TCG TAC GC |
| PDC5-S2 (SEQ ID NO: 8) | AAA ATA CAC AAA CGT TGA ATC ATG AGT TTT ATG TTA ATT AGC ATA GGC CAC TAG TGG ATC TG |
| PDC6-S1 (SEQ ID NO: 9) | TAA ATA AAA AAC CCA CGT AAT ATA GCA AAA ACA TAT TGC CCA GCT GAA GCT TCG TAC GC |
| PDC6-S2 (SEQ ID NO: 10) | TTT ATT TGC AAC AAT AAT TCG TTT GAG TAC ACT ACT AAT GGC ATA GGC CAC TAG TGG ATC TG |
| PDC2-S1 (SEQ ID NO: 11) | ACG CAA CTT GAA TTG GCA AAA TGG GCT TAT GAG ACG TTC CCA GCT GAA GCT TCG TAC GC |
| PDC2-S2 (SEQ ID NO: 12) | AGC CTG TGT TAC CAG GTA AGT GTA AGT TAT TAG AGT CTG GGC ATA GGC CAC TAG TGG ATC TG |

At p. 40, line 15 to p. 41, line 4:

A double deletant strain *Klpdc1Δ/Klpda1Δ* was selected from the haploid segregant population of a diploid strain obtained by crossing strain MW341-5/*Klpdc1Δ* (MATα, *lac4-8, leu2, lysA1-1, uraA1-1, Klpdc1::URA3*; obtained as previously described in Bianchi et. al, 1996, Mol. Microbiol. 19 (1), 27-36, Destruelle et al., submitted) with strain CBS2359/*Klpda1Δ* (MATa, *URA3-48, Klpda1::Tn5BLE)* Deletion of the *PDA1* gene, encoding for the pyruvate dehydrogenase complex E1-alpha subunit (EC.1.2.4.1) (the DNA sequence has been obtained by Steensma H.Y.; Faculty of Mathematics and Natural Sciences, Clusius Laboratory, Leiden, The ~~Netherland~~ Netherlands- the DNA sequence is also available at the accession ~~n.~~ no. AF023920 of the Genbank Sequence Database provided by the National Center of Biotechnology -NCBI- Web site: ~~http://www.ncbi.nlm.nih.gov/~~ http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=2558902, accessed February 16, 2004, reporting a sequence first published by Zeeman, A.M., Luttik, M.A., Thiele, C., van Dijken, J.P., Pronk, J.T. and Steensma, H.Y., "Inactivation of the Kluyveromyces lactis KlPDA1 gene leads to loss of pyruvate dehydrogenase activity, impairs growth on glucose and triggers aerobic alcoholic fermentation," *Microbiology* 144 (Pt 12), 3437-3446 (1998)), in the yeast strain CBS2359 has been obtained following the classical PCR approach and yeast transformation described throughout the text. We used the marker Tn5Ble (Gatignol et al., Gene, 91:35, 1990) conferring phleomycin resistance, as a marker of the integration.

At p. 53, Table C (next page):

Table C

Overview of the cultivation with *S. cerevisiae* (CENPK113 Δpdc1 Δpdc5 Δpdc6 [pLC5-KanMX]

| Time [h] | pH | $OD_{660}$ | glucose [$g.l^{-1}$] | ethanol [$g.l^{-1}$] | lactate [$g.l^{-1}$] |
|---|---|---|---|---|---|
| 0.0 | 5.74 | 0.82 ± 0.01 | 92 ± 1 | | 0.171 ± 0.005 |
| 10.0 | 5.16 | 1.185 ± 0.02 | 93 ± 1 | | 0.715 ± 0.0 |
| 23.5 | 4.61 | 1.28 ± 0.03 | 94 ± 2 | | 1.76 ± 0.1 |
| 49.25 | 4.05 | 1.36 ± 0.03 | 92.8 ± 0.8 | | 3.614 ± 0.005 |
| 73.0 | 3.79 | 1.27 ± 0.03 | 89.0 ± 0.7 | | 5.17 ± 0.01 |
| 106.0 | 3.60 | 1.25 ± 0.02 | 80 ± 2 | | 6.84 ± 0.06 |
| 122.5 | 3.57 | ~~81.24 ± 0.06~~<br>1.23 ± 0.05 | ~~1.23 ± 0.05~~<br>81.24 ± 0.06 | 0 | 7.596 ± 0.006 |
| 167.0 | 3.43 | ~~75 ± 1~~<br>1.17 ± 0.08 | ~~1.17 ± 0.08~~<br>75 ± 1 | | 8.5 ± 0.2 |